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ARTICLE

Heterozygous deletion of *Seipin* in islet beta cells of male mice has an impact on insulin synthesis and secretion through reduced PPARγ expression

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Abstract

Aims/hypothesis Berardinelli–Seip congenital lipodystrophy type 2 (BSCL2) is an autosomal recessive disorder characterised by lipodystrophy and insulin resistance. BSCL2 is caused by loss-of-function mutations in the *Seipin* gene (also known as *Bscl2*). Deletion of this gene in mice induces insulin resistance, glucose intolerance and a loss of adipose tissue. This study evaluated the effects of genetic deletion of *Seipin* on islet beta cell function.

Methods We examined seipin expression in islet cells and measured glucose profiles, insulin synthesis, glucose-stimulated insulin secretion (GSIS), islet expression of peroxisome proliferator-activated receptor γ (PPARγ), levels of *Pdx-1*, *Nkx6.1*, *Glut2* (also known as *Slc2a2*) and proinsulin mRNA, nuclear translocation of pancreatic duodenal homeobox 1 (PDX-1), islet numbers, and beta cell mass and proliferation in male and female *Seipin*-knockout homozygous ($Seipin^{-/-}$) and heterozygous ($Seipin^{+/-}$) mice.

Results Male and female $Seipin^{-/-}$ mice displayed glucose intolerance, insulin resistance, hyperinsulinaemia and a lack of adipose tissue. By contrast, male but not female $Seipin^{+/-}$ mice showed glucose intolerance without adipose tissue loss or insulin resistance. Seipin was highly expressed in islet beta cells in wild-type mice. Expression of islet PPARγ was reduced in male $Seipin^{-/-}$ and $Seipin^{+/-}$ mice but not in female $Seipin^{-/-}$ or $Seipin^{+/-}$ mice. Treatment of male $Seipin^{+/-}$ mice with rosiglitazone corrected the glucose intolerance. Male $Seipin^{+/-}$ mice displayed a decrease in islet insulin concentration and GSIS with low expression of *Pdx-1*, *Nkx6.1*, *Glut2* and proinsulin, and a decline in PDX-1 nuclear translocation; these changes were rescued by rosiglitazone administration. Male $Seipin^{-/-}$ mice showed obvious, but rosiglitazone-sensitive, increases in islet insulin concentration, islet number and beta cell mass and proliferation, with a notable decline in GSIS. Ovariectomised female $Seipin^{+/-}$ mice displayed glucose intolerance and deficits in insulin synthesis and secretion, with a decline in islet PPARγ level; these deleterious effects were reversed by administration of oestradiol or rosiglitazone.

Jianwei Xiong and Peng Sun contributed equally to this work.

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Research in context

What is already known about this subject?

- Berardinelli–Seip congenital lipodystrophy type 2 (BSCL2) is caused by a mutation in the *Seipin* gene and is characterised by lipodystrophy, glucose intolerance and insulin resistance
- *Seipin* deletion in mice and rats causes glucose intolerance and insulin resistance

What is the key question?

- What are the effects of *Seipin* gene deletion on islet beta cell function?

What are the new findings?

- Seipin is highly expressed in islet beta cells of wild-type mice
- *Seipin* deletion in mouse islet beta cells impacts on insulin synthesis and secretion through reducing PPARγ expression, leading to glucose intolerance
- Oestrogen rescues dysfunction of the islet beta cells caused by *Seipin* deletion by correcting PPARγ expression

How might this impact on clinical practice in the foreseeable future?

- These findings provide new perspectives for therapies to treat glucose intolerance in people with BSCL2; it is possible that a single allele mutation in the *Seipin* gene may be a susceptibility factor for beta cell dysfunction in humans

Conclusions/interpretation Heterozygous deletion of *Seipin* in islet beta cells impacts on insulin synthesis and secretion through reduced PPARγ expression. This leads to glucose intolerance and is relieved by oestradiol, which rescues PPARγ expression.

Keywords Berardinelli · Islet beta cell · Oestrogen · Peroxisome proliferator-activated receptor γ (PPARγ) · Seip congenital lipodystrophy type 2 (BSCL2) · Seipin

Abbreviations

BSCL2	Berardinelli–Seip congenital lipodystrophy type 2
ER	Endoplasmic reticulum
GSIS	Glucose-stimulated insulin secretion
PDX-1	Pancreatic duodenal homeobox 1
PPARγ	Peroxisome proliferator-activated receptor γ
PPRE	Peroxisome proliferator-activated receptor γ response element
RT-qPCR	Reverse-transcription quantitative PCR
WT	Wild-type

Introduction

Berardinelli–Seip congenital lipodystrophy type 2 (BSCL2) is an autosomal recessive disorder characterised by a near total lack of adipose tissue, together with severe insulin resistance, glucose intolerance, liver steatosis and hypertriacylglycerolaemia [1]. BSCL2 is caused by loss-of-function mutations in the *Seipin* gene (also known as *Bscl2*) [2, 3]. *Seipin*-knockout ($Seipin^{-/-}$) mice recapitulate many aspects of human BSCL2, such as a dramatic loss of adipose tissue, insulin resistance, glucose intolerance and hepatic steatosis [4].

Depletion of seipin, an exclusive endoplasmic reticulum (ER)-resident *N*-glycosylated protein, has been reported to decrease the generation of peroxisome proliferator-activated receptor γ (PPARγ) in murine embryonic fibroblasts, stromal vascular cells [5] and adipose tissue [6, 7]. PPARγ is a transcription factor that is involved in insulin sensitivity, adipocyte differentiation, lipid storage and glucose uptake [8]. Knockdown of seipin reduces the differentiation of adipocytes and this reduction is rescued by the PPARγ agonist pioglitazone [9]. In particular, treatment with PPARγ agonists has been reported to improve insulin sensitivity and glucose tolerance in $Seipin^{-/-}$ mice [6].

PPARγ is expressed in islet beta cells, where it is involved in the beta cell proliferation and apoptosis, and is involved in insulin synthesis and secretion [10]. PPARγ, through increased expression of pancreatic duodenal homeobox 1 (PDX-1), regulates insulin transcription [11]. A 75% loss of PPARγ, using RNA interference, can reduce *Nkx6.1* (also known as *Nkx6-1*, encoding beta cell-specific transcription factor NK6 homeobox 1) and *Glut2* (also known as *Slc2a2*) mRNA levels, and affects glucose-stimulated insulin secretion

(GSIS) [11]. Therefore, if beta cells express seipin protein, a seipin deficiency would be expected to reduce PPARγ and thereby affect insulin synthesis and secretion. The focus of the present study was to evaluate the influence of seipin deficiency on islet beta cell function.

An earlier study [12] reported sex-related downregulation of PPARγ expression in the brain of *Seipin*$^{-/-}$ mice, where administration of oestradiol rescued the reduction in PPARγ expression. These findings suggest a correlation between seipin-reduced PPARγ expression and oestrogen. In humans, BSCL2 is a recessive disease. Seipin-deficient homozygous (*Seipin*$^{-/-}$) rats exhibit a reduction in body weight but heterozygous (*Seipin*$^{+/-}$) rats do not display changes in body weight [13]. Windpassinger et al. [14] reported that heterozygous missense mutations in the *Seipin* gene led to distal hereditary motor neuropathy and Silver syndrome. However, whether the heterozygous deletion of *Seipin* affects metabolism and glucose homeostasis remains unclear. In this study, we used 3-month-old male and female *Seipin*$^{-/-}$ and *Seipin*$^{+/-}$ mice to examine the influence of the homozygous and heterozygous deletion of *Seipin* in glucose homeostasis and insulin synthesis and secretion, and explored the underlying molecular mechanisms.

Methods

Generation of *Seipin* null mice and experimental design All animal experiments were approved by the Institutional Animal Care and Ethical Committee of the Nanjing Medical University. The *Seipin*-knockout mice were generated and their genotypes identified as described previously [4]. Eight-week-old animals were used in this study. The mice were maintained under constant environmental conditions (23 ± 2°C, humidity of 55 ± 5%, and a 12:12 h light/dark cycle) with free access to food (a standard laboratory chow) and water. A glucose-lowering drug of the thiazolidinone class, rosiglitazone (Sigma-Aldrich, St Louis, MO, USA) was orally administered daily [15]. Six days after ovariectomy [16], oestradiol (5 μg/kg per day; β-Estradiol, E2758, Sigma-Aldrich) was injected subcutaneously [17]. In this study, male wild-type (WT) mice ($n = 20$), female WT mice ($n = 28$), male *Seipin*$^{-/-}$ mice ($n = 26$), male *Seipin*$^{+/-}$ mice ($n = 34$), female *Seipin*$^{-/-}$ mice ($n = 20$) and female *Seipin*$^{+/-}$ mice ($n = 44$) were randomly divided into three experimental groups. The first group was used to examine serum leptin and insulin, plasma glucose, and glucose and insulin tolerance, followed by islet immunostaining ($n = 6$ per experimental group). In the second group, islets were isolated to examine insulin secretion and mRNA expression ($n = 8$ per experimental group). In the third group, isolated islets were treated with rosiglitazone or oestradiol, and insulin secretion and mRNA expression were examined ($n = 8$ per experimental group).

Measurement of plasma glucose and insulin After mice were fasted for 6 h, blood samples were obtained from the tail vein to measure the levels of fasting plasma glucose by the glucose oxidase method (Contour Glucometer; Bayer, Toronto, ON, Canada). Orbital blood was obtained and the level of fasting serum insulin was analysed by an ELISA (Mouse Insulin; Mercodia, Winston-Salem, NC, USA). The intra- and inter-assay CV for insulin was 1.67% and 2.25%, respectively.

ITT After mice were fasted for 6 h, human recombinant insulin (1 IU/kg; Novolin-R; Novo Nordisk, Plainsboro, NJ, USA) was injected intraperitoneally. Blood (5 μl) was obtained from the tail tip before insulin injection and at 15, 30, 60 and 120 min after insulin injection [18]. ITT curves were constructed for the 0–120 min experimental time window and the AUC was calculated.

GTT and glucose-stimulated insulin secretion For in vivo determination of GSIS, D-glucose (1 g/kg) was injected intraperitoneally after mice had been fasted for 6 h. Blood samples were collected from the sublingual vein at 0, 2, 5, 10, 30, 60 and 120 min after glucose loading and plasma glucose and insulin levels were measured [19]. For in vitro determination of insulin secretion, islets (isolated from mice as previously described [20]) were perfused with 5 mmol/l glucose for 60 min to obtain the basal insulin release and then treated with 25 mmol/l glucose for another 1 h to determine GSIS [20].

Immunohistochemistry and immunofluorescence Mice were euthanised with intraperitoneal injection of pentobarbital (3 mg/100 ml) and the image of viscera in each mouse was taken by a stereoscopic microscope (RWD Life Science, Shenzhen, China). The visceral fat (perirenal, mesenteric and epididymal) was removed and weighed to calculate visceral fat to body weight ratio. The pancreases were removed and fixed in Bouin's fluid for making paraffin sections. Rabbit anti-seipin antibody was kindly provided by J. Sha (Nanjing Medical University); other antibodies (diluted in PBS with 1% BSA) are listed in electronic supplementary material (ESM) Table 1. All antibodies were validated using positive controls. Immunohistochemical staining was observed using a DP70 microscope (Olympus Optical, Tokyo, Japan) and immunofluorescence staining was observed using a laser scanning confocal microscope (FV1000; Olympus). The immunoreactivity of insulin was calculated by integral absorbance (hue: 0–30; saturation: 30–200; intensity: 20–210) corrected for islet area using ImageJ software (https://imagej.nih.gov/ij/, National Institutes of Health, USA). Pancreatic beta cell mass was calculated as (islet area/pancreas area) × pancreas weight. The Ki67-positive ratio was calculated as number of Ki67-positive nuclei per islet area. The PDX-1 nuclear translocation ratio was calculated as the number of PDX-1-positive nuclei divided by total number of islet nuclei.

Primary cultured islets, western blotting and reverse-transcription quantitative PCR Mice were anaesthetised with chloral hydrate and then their islets were isolated as previously described [20]. Briefly, collagenase V (1 mg/ml concentration, Sigma-Aldrich) was injected into the pancreas through the common bile duct. The pancreas tissue was then quickly removed and digested to isolate islets. Freshly isolated islets were lysed in RIPA buffer (Beyotime, Shanghai, China) to extract protein or in Trizol (Invitrogen, Carlsbad, CA, USA) to extract total RNA, following the manufacturer's instructions. Western blotting and reverse-transcription quantitative PCR (RT-qPCR) analyses were performed as previously described [21]. For RT-qPCR, after reverse transcription, qPCR analyses were carried out in a LightCycler 480 (Roche Diagnostic, Branchburg, NJ, USA). The primers were synthesised by Invitrogen and the primer sequences are listed in ESM Table 2. The *Gadph* gene was used as an internal control.

Statistical analysis All experimenters were blind to group assignment and outcome assessment. Data are expressed as the mean ± SE. All statistical analyses were performed using SPSS software, version 18.0 (SPSS, Chicago, IL, USA). Differences among the means were analysed using Student's *t* test or ANOVA with or without repeated measures, followed by the Bonferroni post hoc test where appropriate. Differences were considered statistically significant at $p < 0.05$.

Results

Seipin deficiency alters insulin sensitivity and glucose homeostasis Mean body weight did not differ significantly between the groups of mice at 3 months of age ($p > 0.05$) (Fig. 1a). *Seipin*$^{-/-}$ mice of both sexes displayed an obvious reduction in their visceral fat (Fig. 1b) but the *Seipin*$^{+/-}$ mice did not. When compared with WT mice, the ratio of visceral fat weight to body weight was reduced by approximately 40–50% in male *Seipin*$^{-/-}$ mice ($p < 0.01$) and female *Seipin*$^{-/-}$ mice ($p < 0.05$) (Fig. 1c), along with a decline in the level of serum leptin (male *Seipin*$^{-/-}$ mice, $p < 0.01$; female *Seipin*$^{-/-}$ mice, $p < 0.05$) (Fig. 1d), but the ratio of visceral fat weight to body weight was the same in male *Seipin*$^{+/-}$, female *Seipin*$^{+/-}$ and WT mice ($p > 0.05$). GTT analysis revealed higher plasma glucose levels at 15, 30 and 60 min after the glucose challenge in male *Seipin*$^{-/-}$ mice (15 min, $p < 0.01$; 30–60 min, $p < 0.05$), female *Seipin*$^{-/-}$ mice (30/120 min, $p < 0.05$; 60 min, $p < 0.01$) and male *Seipin*$^{+/-}$ mice (30–60 min, $p < 0.05$) than in WT mice but the levels did not differ between female *Seipin*$^{+/-}$ mice and WT mice ($p > 0.05$) (Fig. 1e,f). The AUC for the GTT was larger for male *Seipin*$^{-/-}$ ($p < 0.05$), female *Seipin*$^{-/-}$ ($p < 0.05$) and male *Seipin*$^{+/-}$ mice ($p < 0.05$) than for the WT mice (Fig. 1g). The response to an insulin injection during the ITT was significantly reduced in male *Seipin*$^{-/-}$ (15/60 min, $p < 0.01$; 30 min, $p < 0.05$) and female *Seipin*$^{-/-}$ mice (60 min, $p < 0.01$) when compared with WT mice, whereas the response in male *Seipin*$^{+/-}$ and female *Seipin*$^{+/-}$ mice did not differ from that of the WT mice ($p > 0.05$) (Fig. 1h,i). The AUC of the ITT was increased in both male and female *Seipin*$^{-/-}$ mice ($p < 0.05$) when compared with WT mice (Fig. 1j).

Seipin is selectively expressed in islet beta cells Immunohistochemistry revealed a selective and high level of seipin expression in pancreatic islet cells (Fig. 2a). When compared with WT mice, the seipin immunoreaction in *Seipin*$^{+/-}$ mice of both sexes was clearly reduced (Fig. 2b). A 30–40% decline was also observed in the level of *Seipin* mRNA in islets isolated from male *Seipin*$^{+/-}$ mice of both sexes ($p < 0.05$) (Fig. 2c). *Seipin* mRNA was not detected in the islets of male *Seipin*$^{-/-}$ mice or female *Seipin*$^{-/-}$ mice ($p < 0.01$). The seipin protein was localised in the cytoplasm of the islet cells. As shown in Fig. 2d, the immunoreaction of seipin was mostly overlapped in the insulin-positive beta cells, rather than in the glucagon-positive alpha cells, pancreatic polypeptide-positive pancreatic polypeptide cells or somatostatin-positive delta cells. The beta cell marker PDX-1 was found in the seipin-positive cells (Fig. 2d). Moreover, the immunoreaction of seipin was colocalised with the ER marker calnexin (Fig. 2d). The specificity of the immunostaining was confirmed by the lack of seipin in the islet beta cells of male *Seipin*$^{-/-}$ mice (Fig. 2e).

Seipin deficiency disrupts insulin secretion Fasting serum insulin levels were higher in *Seipin*$^{-/-}$ mice of both sexes ($p < 0.01$) than in WT mice ($p < 0.01$) (Fig. 3a). Insulin levels were slightly reduced in male *Seipin*$^{+/-}$ mice vs WT mice but the difference did not reach statistical significance; levels did not differ between female *Seipin*$^{+/-}$ mice and WT mice ($p > 0.05$). When compared with WT mice, the integrated absorbance of the insulin immunoreaction (Fig. 3b) was increased in male and female *Seipin*$^{-/-}$ mice (both $p < 0.05$), whereas it was reduced in male *Seipin*$^{+/-}$ mice ($p < 0.05$) and was not altered in female *Seipin*$^{+/-}$ mice ($p > 0.05$) (Fig. 3c). As shown in Fig. 3d,e, the GSIS was measured at 2–5 min (as the first phase) and 10–120 min after glucose injection (as the second phase), respectively. When compared with WT mice, the AUC of the first-phase GSIS in male *Seipin*$^{-/-}$ mice was lower ($p < 0.05$; Fig. 3f), whereas the AUC of the second-phase GSIS was higher ($p < 0.05$; Fig. 3g). By contrast, female *Seipin*$^{-/-}$ mice only displayed an increase in the AUC of the second-phase GSIS in comparison with WT mice ($p < 0.05$). Notably, the AUCs of the first-phase GSIS ($p < 0.01$) and the second-phase GSIS ($p < 0.05$) were reduced in male *Seipin*$^{+/-}$ mice; however, the responses of the female *Seipin*$^{+/-}$ mice did not differ significantly from those of the WT mice ($p > 0.05$).

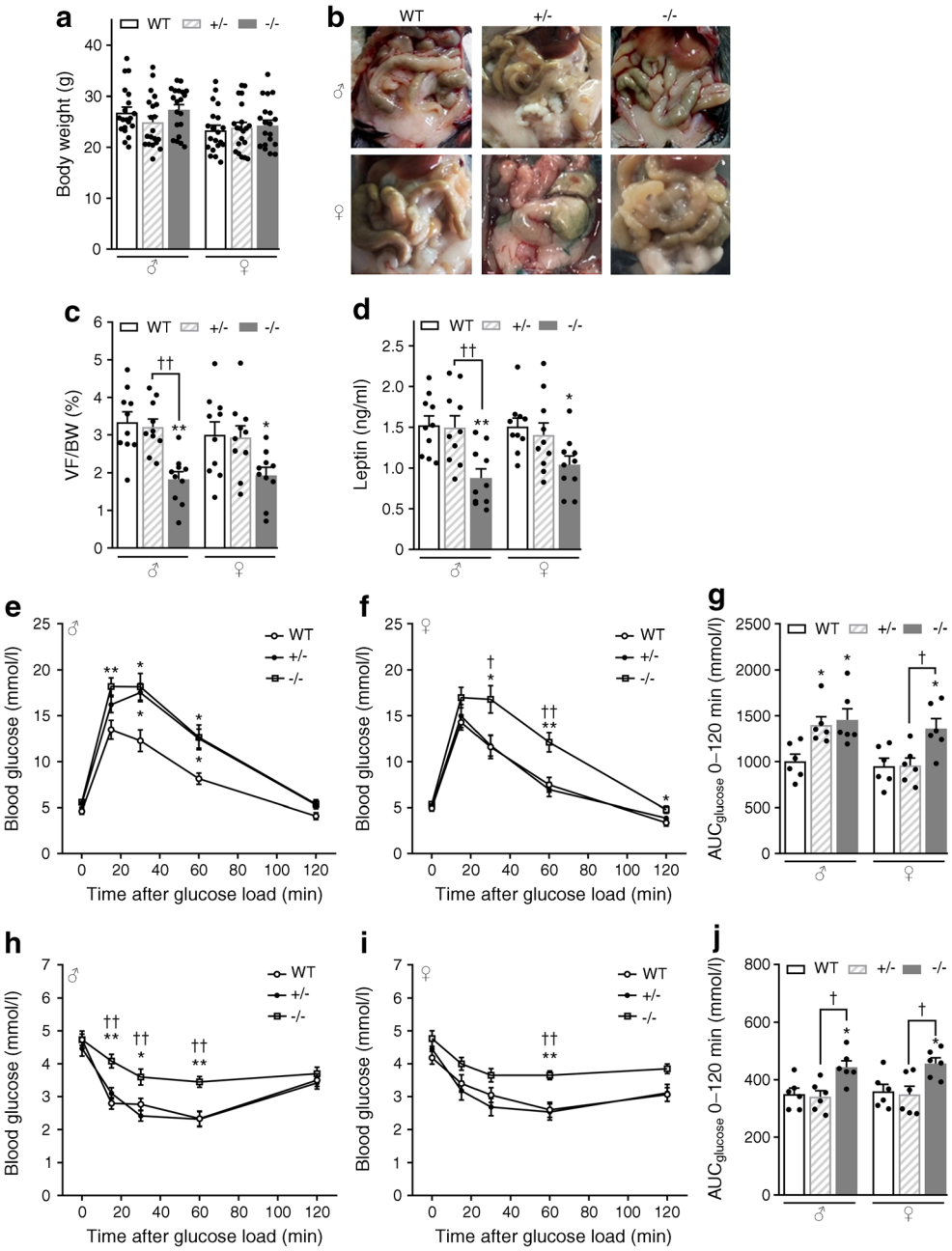

Fig. 1 Seipin deficiency alters insulin sensitivity and glucose homeostasis. (**a**) Body weight of 3-month-old male and female WT, *Seipin*$^{+/-}$ and *Seipin*$^{-/-}$ mice ($n = 20$ per group). (**b**) Representative images of visceral adipose tissue. (**c**, **d**) Ratio of visceral fat weight (VF) to body weight (BW) (**c**) and level of serum leptin (**d**). *$p < 0.05$, **$p < 0.01$ vs WT mice of the same sex; ††$p < 0.01$ vs male *Seipin*$^{+/-}$ mice (one-way ANOVA followed by Bonferroni post hoc test, $n = 10$ per group). (**e**–**g**) IPGTT curve in male (**e**) and female (**f**) mice and AUC of GTT (**g**). *$p < 0.05$, **$p < 0.01$ vs WT mice of the same sex; †$p < 0.05$, ††$p < 0.01$ vs *Seipin*$^{+/-}$ mice of the same sex (one-way ANOVA followed by Bonferroni post hoc test, $n = 6$ per group). (**h**–**j**) IPITT curve in male (**h**) and female (**i**) mice and AUC of ITT (**j**). *$p < 0.05$, **$p < 0.01$ vs WT mice of the same sex; †$p < 0.05$, ††$p < 0.01$ vs *Seipin*$^{+/-}$ mice of the same sex (one-way ANOVA followed by Bonferroni post hoc test, $n = 6$ per group)

In islets isolated from male *Seipin*$^{-/-}$ mice ($p > 0.05$) or male *Seipin*$^{+/-}$ mice ($p > 0.05$), the basal level of insulin secretion was no different from that in WT mice (Fig. 3h). However, after the isolated islets were treated with 25 mmol/l glucose for 60 min, the levels of in vitro GSIS in male *Seipin*$^{+/-}$ mice ($p < 0.01$) and male *Seipin*$^{-/-}$ mice ($p < 0.05$) were lower than those in WT mice.

Seipin deficiency reduces PPARγ expression to alter insulin secretion When compared with the levels in WT mice, the expression levels of *Pparγ* (also known as *Pparg*) mRNA (Fig. 4a) and PPARγ protein (Fig. 4b) were reduced in the islets obtained from male *Seipin*$^{+/-}$ mice (mRNA/protein $p < 0.05$) and male *Seipin*$^{-/-}$ mice (mRNA/protein $p < 0.01$) but were unchanged in female *Seipin*$^{-/-}$ and *Seipin*$^{+/-}$ mice ($p > 0.05$). Notably, the administration of rosiglitazone was able to correct the glucose intolerance in male *Seipin*$^{+/-}$ mice ($p < 0.05$) (Fig. 4c and ESM Fig. 1a) and increased the absorbance of the insulin immunoreaction ($p < 0.05$) (Fig. 4d), the first-phase GSIS ($p < 0.01$) (Fig. 4e) and the second-phase GSIS ($p < 0.01$) (Fig. 4f). In male *Seipin*$^{-/-}$ mice, treatment with rosiglitazone corrected the level of fasting serum insulin ($p < 0.01$) (Fig. 4g), the absorbance of insulin immunoreaction ($p < 0.01$) (Fig. 4d) and the first-phase GSIS ($p < 0.05$) (Fig.

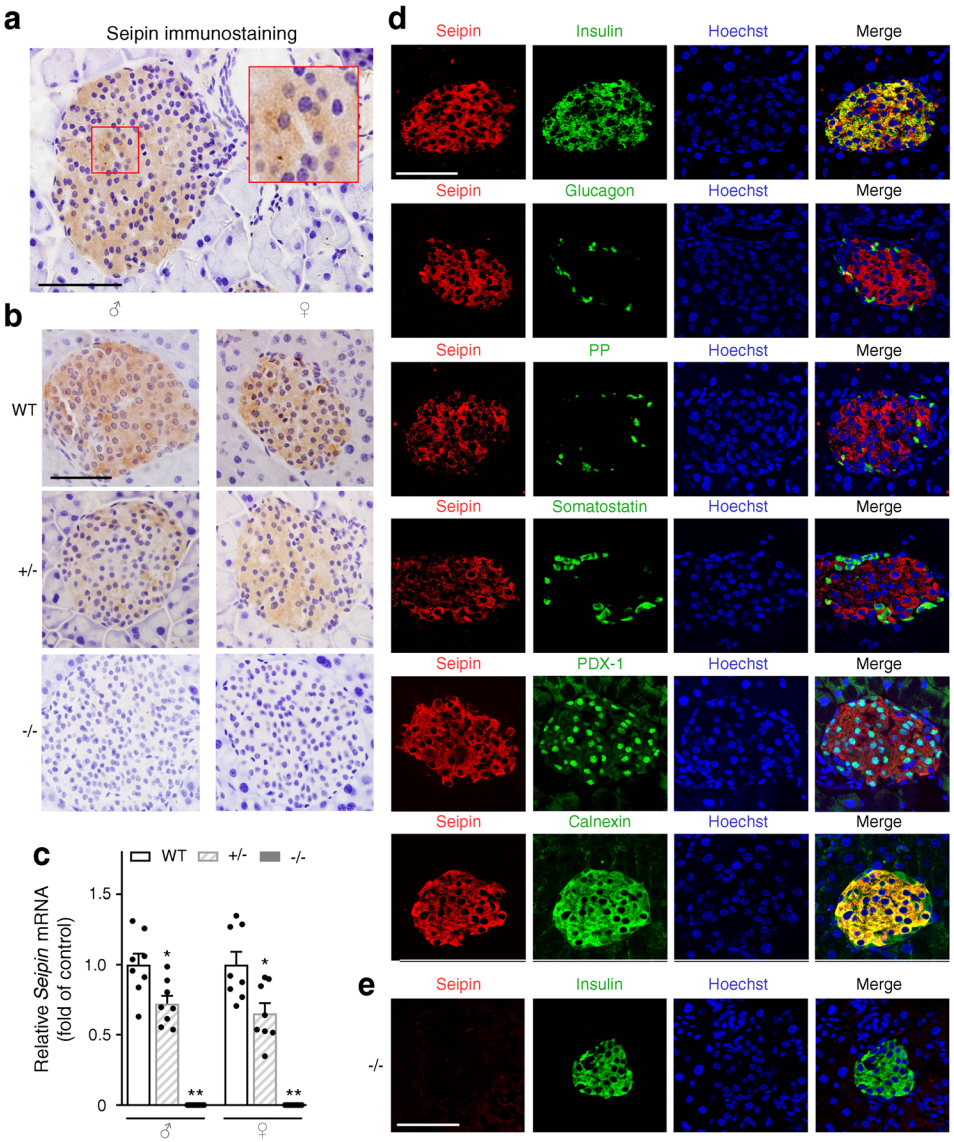

Fig. 2 Seipin is selectively expressed in islet beta cells. (**a**) Representative images of seipin immunostaining (brown) in the pancreas of WT mice. The magnified inset image shows the cytoplasmic distribution of seipin protein. Scale bar, 50 μm. (**b**) Immunostaining for seipin in WT, *Seipin*$^{+/-}$ and *Seipin*$^{-/-}$ mice. Scale bar, 50 μm. (**c**) Levels of *Seipin* mRNA in islets from WT, *Seipin*$^{+/-}$ and *Seipin*$^{-/-}$ mice. Data are expressed as fold of levels in WT mice. *$p < 0.05$, **$p < 0.05$ vs WT mice of the same sex (Student's *t* test, $n = 6$ per group). (**d**) Pancreases from male WT mice were used for double immunofluorescence. Representative images of seipin (red) and insulin, glucagon, pancreatic polypeptide (PP), somatostatin, PDX-1 or calnexin (all green) double immunofluorescence staining. Nuclei were stained with Hoechst 33342 dye (blue). Scale bar, 50 μm. (**e**) Double immunofluorescence staining of seipin (red) and insulin (green) in male *Seipin*$^{-/-}$ mice. Scale bar, 50 μm. All representative images were selected from 6 mice in each group

4e), whereas the treatment with rosiglitazone failed to recover the glucose intolerance ($p > 0.05$) (Fig. 4c and ESM Fig. 1a), the second-phase GSIS ($p > 0.05$) (Fig. 4f) or insulin resistance ($p > 0.05$) (Fig. 4h and ESM Fig. 1b). Moreover, the treatment with rosiglitazone for 24 h corrected GSIS in islets isolated from male *Seipin*$^{+/-}$ or *Seipin*$^{-/-}$ mice (both $p < 0.05$) (Fig. 4i).

Seipin deficiency, through reduced PPARγ expression, suppresses the expression of regulators of insulin synthesis and secretion The mRNA levels of *Pdx-1* (Fig. 5a), *Nkx6.1* (Fig. 5b) and *Glut2* (Fig. 5c) were decreased (all $p < 0.05$) in the islets isolated from male *Seipin*$^{+/-}$ or *Seipin*$^{-/-}$ mice. The levels of proinsulin mRNA were reduced in the islets isolated from male *Seipin*$^{+/-}$ mice ($p < 0.05$) but not male *Seipin*$^{-/-}$ mice ($p > 0.05$) (Fig. 5d). The systemic administration of rosiglitazone for 14 days or treatment of the isolated islets with rosiglitazone for 24 h rescued the decline in the levels of proinsulin ($p < 0.05$), *Pdx-1* ($p < 0.05$), *Nkx6.1* ($p < 0.05$) and *Glut2* mRNA ($p < 0.05$) in male *Seipin*$^{+/-}$ and *Seipin*$^{-/-}$ mice (Fig. 5a–d). Double immunofluorescence staining also confirmed that PDX-1 nuclear translocation in the islets was lower for male *Seipin*$^{+/-}$ mice than for WT mice ($p < 0.01$) (Fig. 5e,f) and was sensitive to rosiglitazone treatment ($p < 0.05$). Similarly, the ratio of PDX-1 nuclear translocation was lower in male *Seipin*$^{-/-}$ mice than in WT mice ($p < 0.01$).

Seipin deficiency, through reduced PPARγ expression, enhances beta cell proliferation Immunohistochemical analyses for insulin (Fig. 6a) revealed increases in the beta cell mass ($p < 0.05$) (Fig. 6b) and the number of islets ($p < 0.05$) (Fig. 6c) in male and female *Seipin*$^{-/-}$ mice but no changes in male or female *Seipin*$^{+/-}$ mice ($p > 0.05$). Consistent with changes in the fasting serum insulin and the absorbance of the insulin

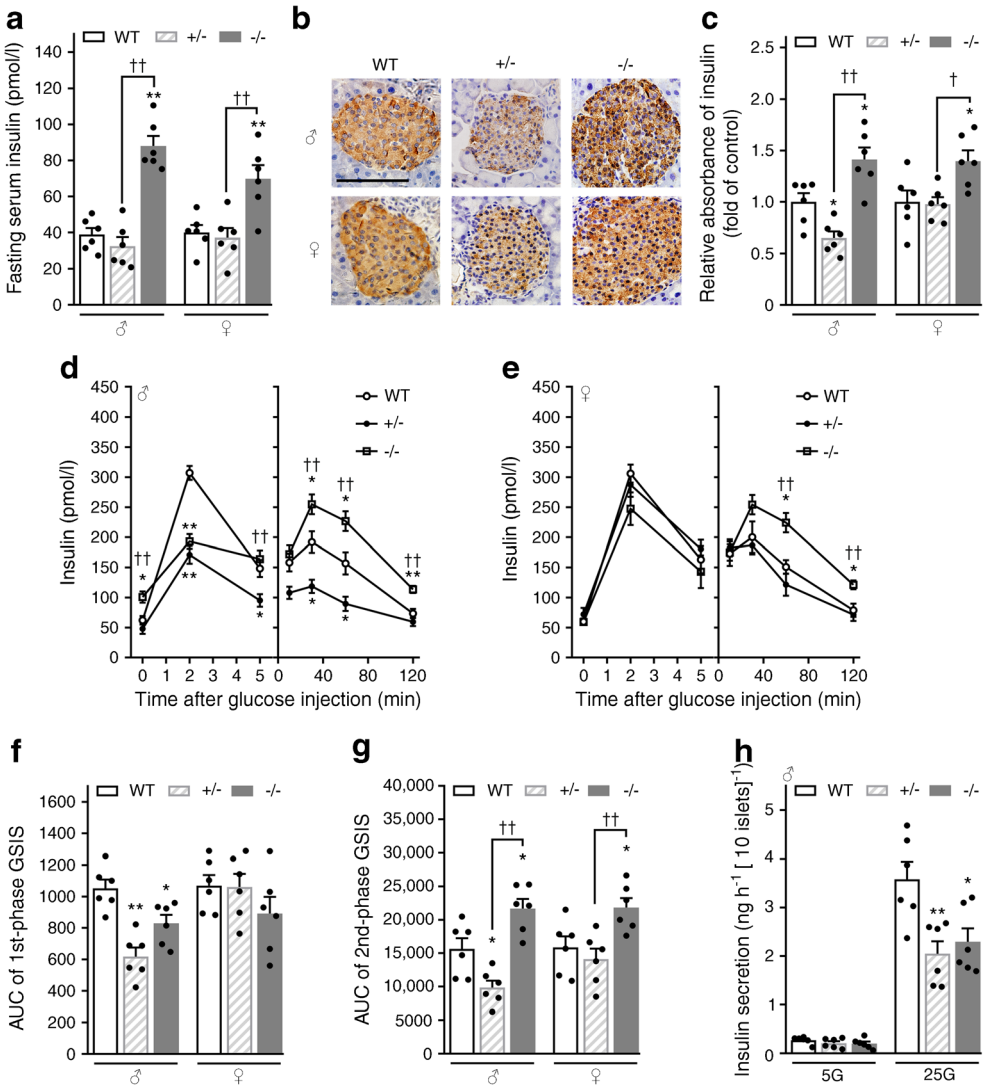

Fig. 3 Seipin deficiency disrupts insulin secretion. (**a**) Fasting serum insulin in WT, *Seipin*$^{+/-}$ and *Seipin*$^{-/-}$ mice. $^{**}p < 0.01$ vs WT mice of the same sex; $^{\dagger\dagger}p < 0.01$ vs *Seipin*$^{+/-}$ mice of the same sex (one-way ANOVA followed by Bonferroni post hoc test, $n = 6$ per group). (**b**) Representative image of insulin immunostaining (brown). Scale bar, 50 μm. The representative images were selected from 6 mice in each group. (**c**) Integrated absorbance of insulin immunoreactions corrected for islet area. Data are expressed as fold of absorbance of insulin immunoreactions for WT mice. $^{*}p < 0.05$ vs WT mice of the same sex; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ vs *Seipin*$^{+/-}$ mice of the same sex (one-way ANOVA, $n = 6$ per group). (**d**, **e**) GSIS in male (**d**) and female (**e**) WT, *Seipin*$^{+/-}$ and *Seipin*$^{-/-}$ mice. (**f**, **g**) AUC of the first-phase GSIS (**f**) and second-phase GSIS (**g**). $^{*}p < 0.05$, $^{**}p < 0.01$ vs WT mice of the same sex; $^{\dagger\dagger}p < 0.01$ vs *Seipin*$^{+/-}$ mice of the same sex (one-way ANOVA, $n = 6$ per group). (**h**) Levels of insulin secretion from isolated islets treated with 5 mmol/l (5G) and 25 mmol/l (25G) glucose. $^{*}p < 0.05$, $^{**}p < 0.01$ vs WT mice of the same sex (one-way ANOVA, $n = 6$ per group)

immunoreaction (Fig. 4e,f), the administration of rosiglitazone for 14 days to male *Seipin*$^{-/-}$ mice restored the islet beta cell mass ($p < 0.05$) and number of islets ($p < 0.05$). A subsequent examination of the beta cell proliferation by double immunohistochemical staining for insulin and Ki67 (Fig. 6d) showed that there was a >30% increase ($p < 0.01$) in the number of insulin/Ki67-positive cells per islet in male *Seipin*$^{-/-}$ mice when compared with WT mice, and this was corrected by the administration of rosiglitazone ($p < 0.01$) (Fig. 6e). By contrast, the number of insulin/Ki67-positive cells did not differ significantly between the male *Seipin*$^{+/-}$ mice and WT mice ($p > 0.05$).

Oestrogen relieves the reduction in PPARγ expression induced by seipin deficiency in female mice The level of *Pparγ* mRNA was reduced in the islets isolated from female *Seipin*$^{+/-}$ mice on the fourth week after ovariectomy ($p < 0.01$ vs ovariectomised WT mice) (Fig. 7a); levels in WT mice were not affected by ovariectomy ($p > 0.05$). The reduction of *Pparγ* mRNA in *Seipin*$^{+/-}$ mice was corrected by the replacement of oestrogen with oestradiol ($p < 0.01$). In addition, ovariectomised *Seipin*$^{+/-}$ mice, but not ovariectomised WT mice, displayed glucose intolerance ($p < 0.05$) (Fig. 7b and ESM Fig. 2a) and a decline in first-phase ($p < 0.01$) (Fig. 7c) and second-phase GSIS ($p < 0.01$) (Fig. 7d) without insulin resistance ($p > 0.05$) (Fig. 7e and ESM Fig. 2b). The administration of rosiglitazone or oestradiol for 14 days to ovariectomised *Seipin*$^{+/-}$ mice improved their glucose tolerance (both $p < 0.05$) (Fig. 7b and ESM Fig. 2a), first-phase GSIS (both $p < 0.01$) (Fig. 7c) and second-phase GSIS (both $p < 0.05$) (Fig. 7d). Similarly, ovariectomy reduced the mRNA levels of *Pdx-1* ($p < 0.01$) (Fig. 7f), *Nkx6.1* ($p < 0.05$) (Fig. 7g), *Glut2* ($p < 0.05$) (Fig. 7h) and proinsulin ($p < 0.05$) (Fig. 7i) in *Seipin*$^{+/-}$ mice; levels were restored by the administration of rosiglitazone (*Pdx-1*, $p < 0.01$; *Nkx6.1*, *Glut2* and *proinsulin*, $p < 0.05$) or oestradiol ($p < 0.05$ for all).

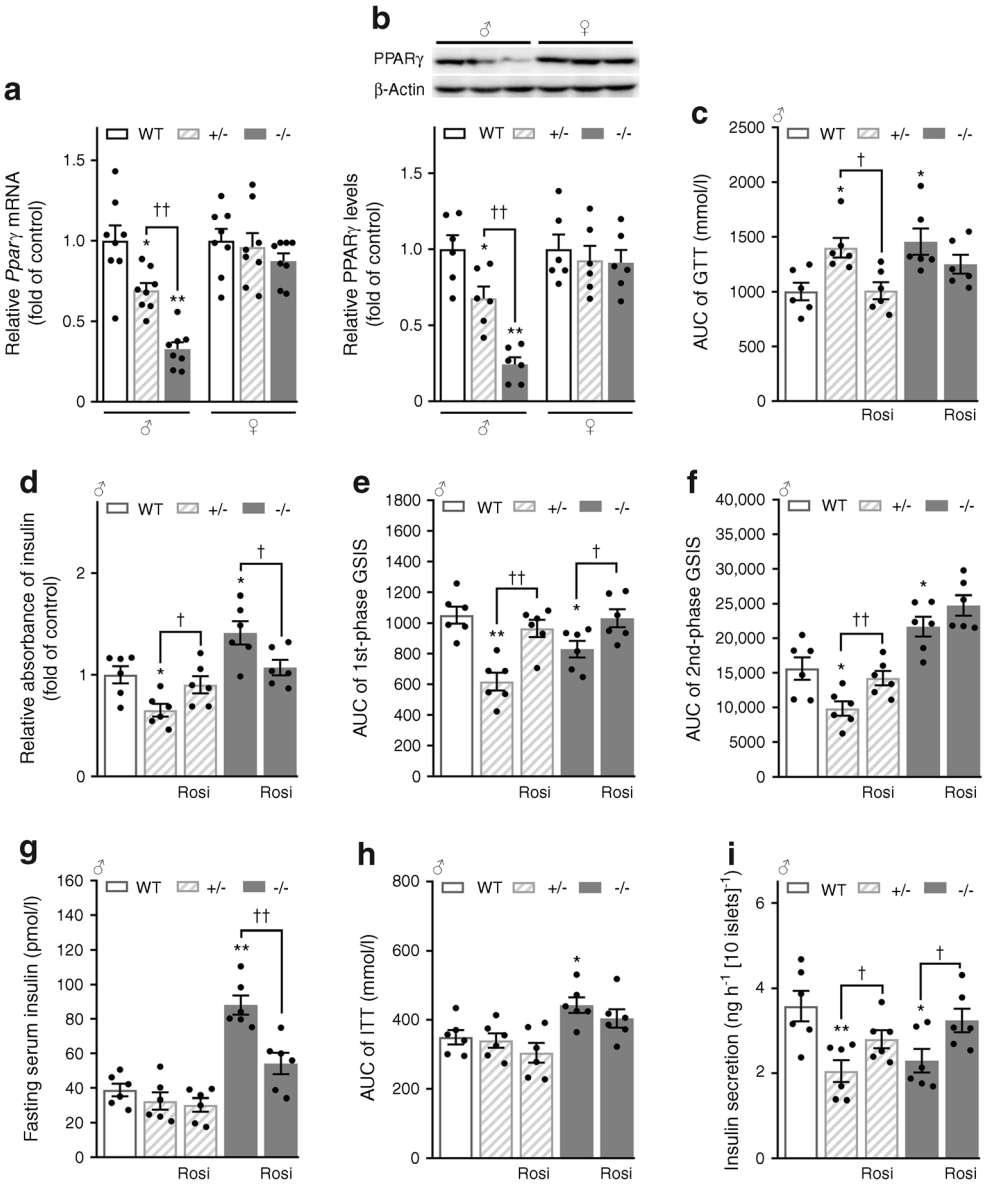

Fig. 4 Seipin deficiency reduces PPARγ expression to alter insulin secretion. (**a**, **b**) Levels of *Pparγ* mRNA (**a**) and PPARγ protein (**b**) in isolated islets. Data are expressed as fold of levels in WT mice. $^{*}p < 0.05$, $^{**}p < 0.01$ vs male WT mice; $^{\dagger\dagger}p < 0.01$ vs male *Seipin*$^{+/-}$ mice (one-way ANOVA, $n = 8$ (**a**) or 6 (**b**) per group). (**c**–**h**) Effects of rosiglitazone (5 mg/kg daily) on AUC of GTT (**c**), integrated absorbance of insulin immunoreactions (**d**), AUC of the first-phase GSIS (**e**) and second-phase GSIS (**f**), fasting serum insulin (**g**) and AUC of ITT (**h**) in male *Seipin*$^{-/-}$ mice and male *Seipin*$^{+/-}$ mice. $^{*}p < 0.05$, $^{**}p < 0.01$ vs WT mice; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ vs no rosiglitazone treatment (one-way ANOVA followed by Bonferroni post hoc test, $n = 6$ per group). (**i**) Insulin secretion from isolated islets from male mice treated with rosiglitazone for 24 h. $^{*}p < 0.05$, $^{**}p < 0.01$ vs WT mice; $^{\dagger}p < 0.05$ vs no rosiglitazone treatment (one-way ANOVA followed by Bonferroni post hoc test, $n = 6$ per group). Rosi, rosiglitazone

Discussion

In the current study we used the model of adult male and female *Seipin*$^{-/-}$ mice and male and female *Seipin*$^{+/-}$ mice and provided, for the first time, the following in vivo and in vitro evidence: (1) seipin is specifically expressed in islet beta cells and (2) heterozygous deletion of *Seipin* in islet beta cells reduces insulin synthesis and secretion, thereby inducing glucose intolerance.

A critical finding in the present study is that seipin deficiency, through its suppression of PPARγ expression, decreases insulin secretion. This conclusion is deduced mainly from the following observations. First, male *Seipin*$^{+/-}$ and *Seipin*$^{-/-}$ mice displayed glucose intolerance with impaired GSIS, and decreased expression of PPARγ in the islets. Second, the decline in first-phase GSIS in male *Seipin*$^{-/-}$ and *Seipin*$^{+/-}$ mice was recovered by the administration of rosiglitazone. Third, administration of rosiglitazone for 14 days could relieve glucose intolerance in the male *Seipin*$^{+/-}$ mice. However, there were conflicting results showing that the treatment with rosiglitazone failed to improve the glucose intolerance and the high second-phase GSIS in male *Seipin*$^{-/-}$ mice; female *Seipin*$^{-/-}$ mice also appeared to be glucose intolerant but their expression of PPARγ in islets was not altered. Although this discrepancy is difficult to be reconciled in the present study, the contradictory phenotype may arise from insulin resistance in *Seipin*$^{-/-}$ mice, because the GTT monitors alteration in blood glucose concentration, so it actually assesses not only insulin secretion but also peripheral glucose disposal over time [22]. Insulin resistance has been reported in adipose tissue *Seipin*-knockout mice [23]. Lipodystrophy causes the deposition of triacylglycerol in the

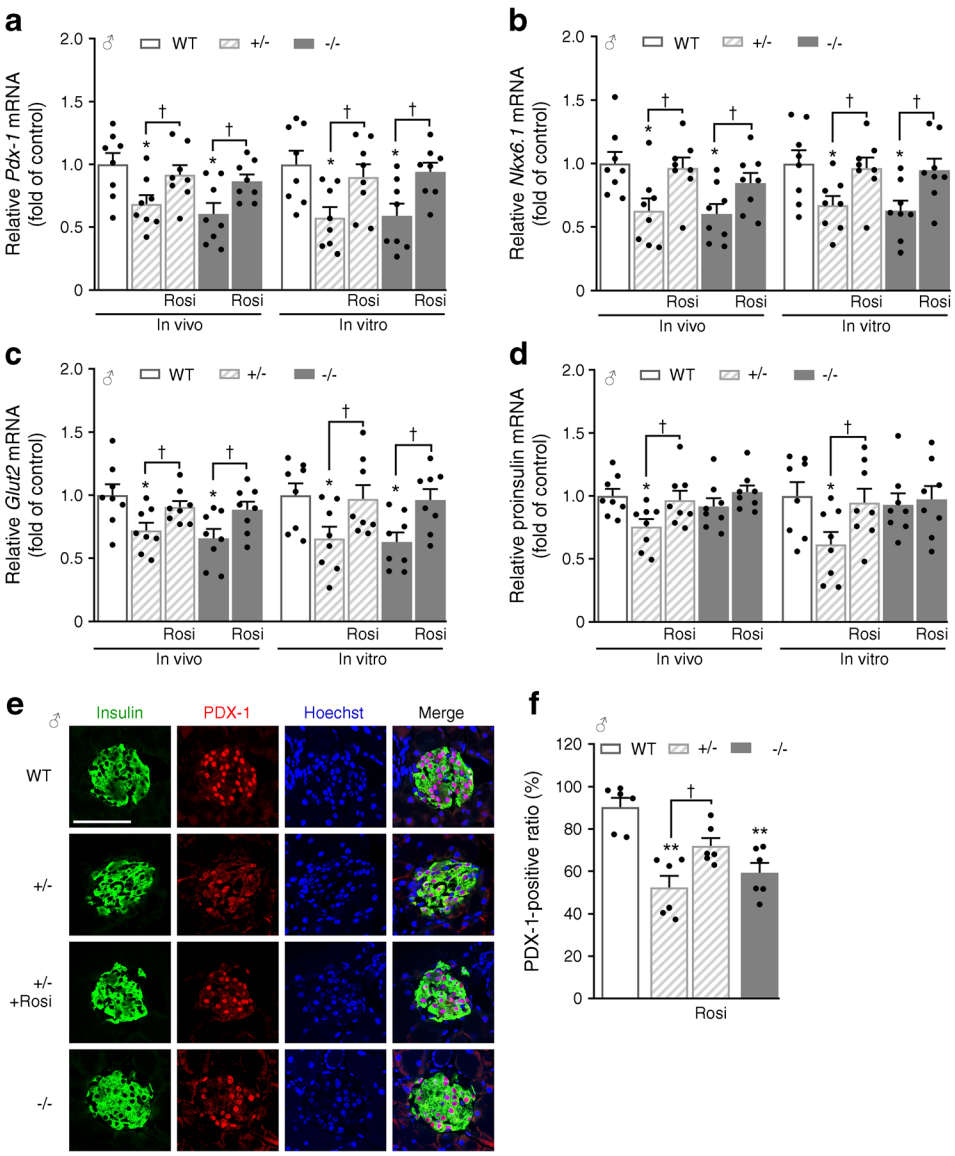

Fig. 5 Seipin deficiency suppresses the expression of regulators of insulin synthesis and secretion in male mice, through its reduction of PPARγ expression. (**a**–**d**) Levels of *Pdx-1*, *Nkx6.1*, *Glut2* and proinsulin mRNA in islets of WT mice and male *Seipin*$^{-/-}$ and *Seipin*$^{+/-}$ mice treated with rosiglitazone (in vivo), or in isolated islets treated with rosiglitazone (in vitro). Data are expressed as fold of WT mice. *$p < 0.05$ vs WT mice; †$p < 0.05$ vs no rosiglitazone treatment (two-way ANOVA, $n = 8$ per group). (**e**) Representative images of insulin (green) and PDX-1 (red) double immunofluorescence staining. Nuclei were stained with Hoechst 33342 dye (blue). Scale bar, 50 μm. The representative images were selected from 6 mice in each group. (**f**) Ratio of PDX-1 nuclear translocation in islets quantified from immunofluorescence images as in (**e**). **$p < 0.01$ vs WT mice; †$p < 0.05$ vs no rosiglitazone treatment (two-way ANOVA, $n = 6$ per group). Rosi, rosiglitazone

liver, leading to hepatic steatosis and insulin resistance. In addition, hepatic insulin signalling is impaired in *Seipin*$^{-/-}$ mice [24]. Low expression of the insulin receptor and insulin receptor substrate 1/2 in *Seipin*$^{-/-}$ mice might produce the hepatic insulin resistance [25].

Many studies demonstrate the effects of PPARγ on the insulin secretion of islet beta cells [26, 27]. In one, PPARγ was found to regulate the transcriptional activity of target genes by forming a heterodimer with retinoid X receptor and binding to a specific PPARγ response element (PPRE) sequence within the promoter region [28]. PPRE has been identified in the 5′ regulatory region of the *Pdx-1* gene, and PPRE mutations dramatically reduce the promoter activity of the *Pdx-1* gene [29]. The expression of PDX-1 in the adult pancreas is generally restricted to islet beta cells (~91%) [30], where it enhances the expression of proinsulin and GLUT2, a glucose transporter expressed in beta cells [31]. Moibi et al. [11] reported that deletion of PPARγ decreases the levels of *Pdx-1* and *Glut2* mRNA. Indeed, the transcript levels of *Pdx-1* and *Nkx6.1*, and ratios of PDX-1 nuclear translocation were significantly reduced in the islets of male *Seipin*$^{+/-}$ and *Seipin*$^{-/-}$ mice, associated with a decline in the expression level of *Glut2*. In addition, seipin is predicted to span the ER membrane twice, with both N- and C-terminals in the cytoplasm and a large luminal loop [32]. Indeed, we observed that the seipin in islet beta cells was colocalised with the ER marker calnexin. Missense mutations (N88S and S90 L) of *Seipin* have been reported to activate the unfolded protein response and induce ER stress [33]. However, the expression levels of C/EBP homologous protein (CHOP) or GRP78 protein, the crucial ER stress markers, are unchanged in the brains of male *Seipin*$^{-/-}$

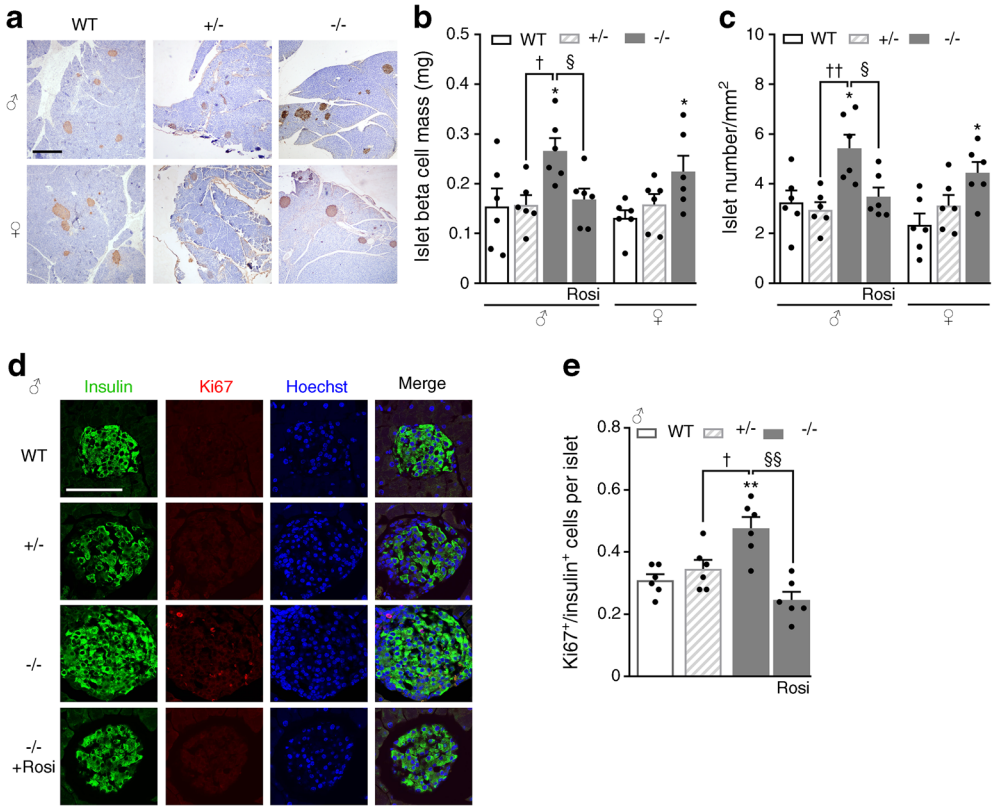

Fig. 6 Seipin deficiency enhances beta cell proliferation, through reduced PPARγ expression. (**a**) Representative images of insulin-positive (brown) beta cells in WT mice, *Seipin*$^{+/-}$ mice and *Seipin*$^{-/-}$ mice. Scale bar, 300 μm. The representative images were selected from 6 mice in each group. (**b**, **c**) Beta cell mass (**b**) and islet number corrected for tissue area (**c**) quantified from immunostaining images as in (**a**). *$p < 0.05$ vs WT mice of the same sex; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ vs male *Seipin*$^{+/-}$ mice; $^{\S}p < 0.05$ vs no rosiglitazone treatment (two-way ANOVA, $n = 6$ per group). (**d**) Representative images of insulin (green) and Ki67 (red) double immunofluorescence staining in the islets of male mice. Nuclei were stained with Hoechst 33342 dye (blue). Scale bar, 50 μm. (**e**) Quantification of immunofluorescence images as in (**d**). Bars indicate the group means of Ki67^{+} cell numbers per islet. **$p < 0.01$ vs WT mice; $^{\dagger}p < 0.05$ vs *Seipin*$^{+/-}$ mice; $^{\S\S}p < 0.01$ vs no rosiglitazone treatment (two-way ANOVA, $n = 6$ per group). Rosi, rosiglitazone

mice [12, 34] or in islets isolated from male *Seipin*$^{-/-}$ mice (data not shown).

Another important finding in the present study is that seipin deficiency, through reducing PPARγ in islet beta cells, impedes insulin synthesis and leads to glucose intolerance. This idea is supported by the decreased insulin synthesis and secretion seen in male *Seipin*$^{+/-}$ mice that was recovered by the administration of rosiglitazone. PDX-1, as a predominant binding factor, regulates insulin gene transcription in pancreatic beta cells [35] and augments the expression of proinsulin to enhance beta cell function and survival rate [36]. An earlier study reported that decreased PDX-1 expression level is related to beta cell mass reduction [31]. Inconsistently, male *Seipin*$^{-/-}$ mice exhibited hyperinsulinaemia with notable increases in insulin content and second-phase GSIS. Furthermore, the islet numbers and the beta cell mass were increased in male *Seipin*$^{-/-}$ mice, and this increase was accounted for by an increase in the proliferation of beta cells. Previous studies reported that the treatment of male *Seipin*$^{-/-}$ mice with rosiglitazone (0.3 mg/g diet) or thiazolidinediones for 9–10 weeks improved insulin resistance [6, 37]. We found that although administration of rosiglitazone (5 mg/kg daily) to male *Seipin*$^{-/-}$ mice for 14 days could correct the beta cell hyperplasia and the excessive synthesis of insulin, it did not reverse the insulin resistance. Mice with a beta cell-specific ablation of PPARγ show beta cell hyperplasia and increased beta cell mass [10]. Thus, it is highly likely that the deficits in the anti-proliferative property of PPARγ [38] in male *Seipin*$^{-/-}$ mice induce beta cell hyperplasia that in turn causes the hyperinsulinaemic status. The level of *PPARγ* mRNA in male *Seipin*$^{-/-}$ mice was reduced by approximately 70%, a greater reduction than that seen in male *Seipin*$^{+/-}$ mice (−30%). Thus, one simple explanation might be that a serious decline in PPARγ expression is critical for triggering the over-proliferation of islet beta cells. On the other hand, leptin deficiency is associated with hyperinsulinaemia in both mice and humans [39, 40]. The lack of adipose tissue, which leads to reduced serum leptin levels in *Seipin*$^{-/-}$ mice of both sexes, may enhance insulin synthesis. It was noted that male *Seipin*$^{-/-}$ mice did not show changes in the level of proinsulin mRNA, although the PDX-1 expression was reduced. One possibility is that the increase in insulin synthesis is a potential contributor to the beta cell compensatory response to

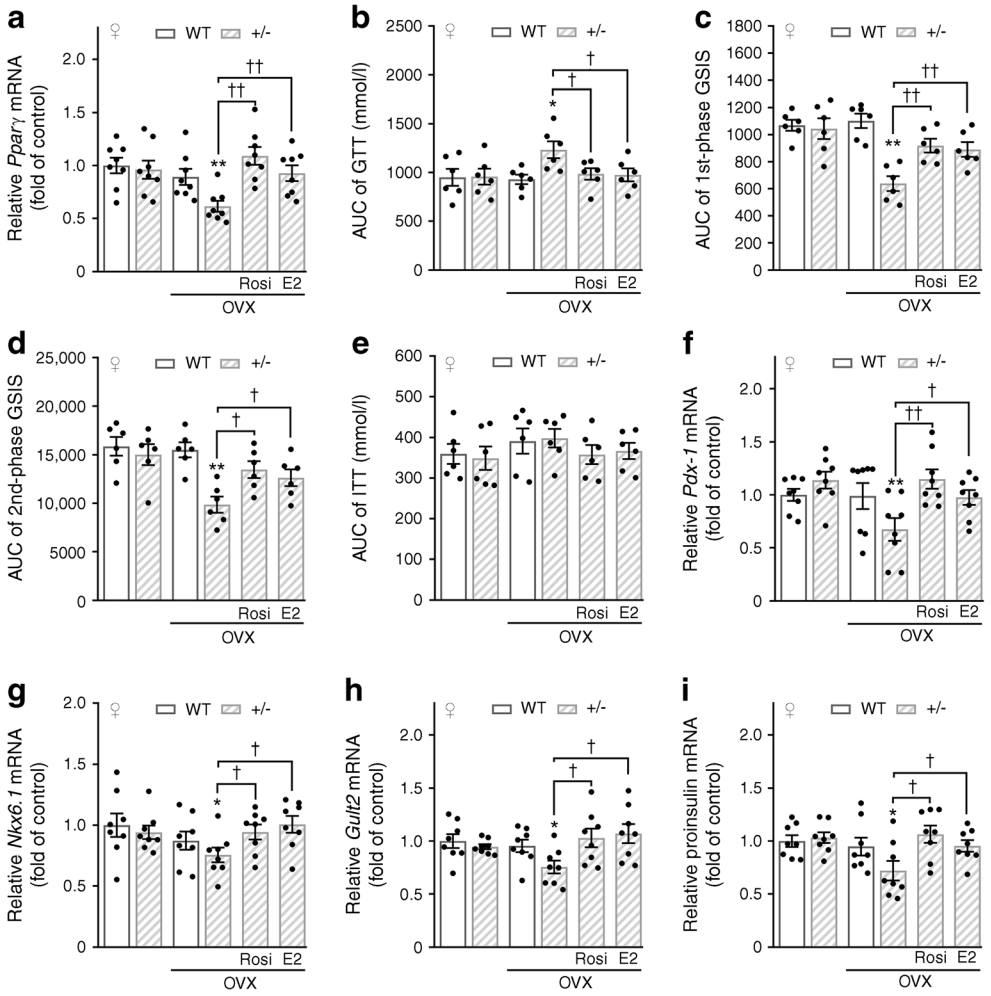

Fig. 7 Oestrogen replacement relieves the reduction in PPARγ expression induced by seipin deficiency in ovariectomised female mice. (**a**) Levels of *Pparγ* mRNA in islets from unoperated and ovariectomised WT mice, unoperated and ovariectomised *Seipin*$^{+/-}$ mice and ovariectomised *Seipin*$^{+/-}$ mice treated with rosiglitazone or oestradiol. Data are expressed as fold of levels in unoperated WT mice. $^{**}p < 0.01$ vs unoperated WT mice; $^{\dagger\dagger}p < 0.01$ vs untreated ovariectomised *Seipin*$^{+/-}$ mice (two-way ANOVA, $n = 8$ per group). (**b**–**e**) Changes in AUC of GTT (**b**), AUC of first-phase GSIS (**c**), AUC of second-phase GSIS (**d**) and AUC of ITT (**e**) in mice treated as in (**a**). $^{*}p < 0.05$ and $^{**}p < 0.01$ vs unoperated WT mice; $^{\dagger}p < 0.05$ and $^{\dagger\dagger}p < 0.01$ vs untreated ovariectomised *Seipin*$^{+/-}$ mice (two-way ANOVA, $n = 6$ per group). (**f**–**i**) Levels of *Pdx-1* (**f**), *Nkx6.1* (**g**), *Glut2* (**h**) and proinsulin mRNA (**i**) in islets from mice treated as in (**a**). Data are expressed as fold of levels in unoperated WT mice. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs unoperated WT mice; $^{\dagger}p < 0.05$ and $^{\dagger\dagger}p < 0.01$ vs untreated ovariectomised *Seipin*$^{+/-}$ mice (two-way ANOVA, $n = 8$ per group). E2, oestradiol; OVX, ovariectomised mice; Rosi, rosiglitazone

insulin resistance [41]. Seipin deficiency may elevate the activity of glycogen synthase kinase-3β (GSK-3β) and the levels of IL-6 and TNF-α by reducing PPARγ expression [42]. Thus, further studies are required to explore the mechanisms underlying seipin deficiency-enhanced proliferation of islet beta cells.

Unlike the male *Seipin*$^{+/-}$ mice, female *Seipin*$^{+/-}$ mice did not show glucose intolerance or decreased insulin synthesis and secretion. Notably, oestrogen deprivation following ovariectomy caused glucose intolerance and deficits in insulin synthesis and secretion without insulin resistance in the female *Seipin*$^{+/-}$ mice but not in WT mice. Oestrogen deprivation has been reported to reduce PPARγ expression in aortic tissue [43]. We observed that ovariectomy induced a decline in PPARγ expression in the islets of *Seipin*$^{+/-}$ mice but not WT mice; this decline responded to oestrogen replacement therapy. Zhou et al. [12] reported that treatment of male *Seipin*$^{-/-}$ mice with oestradiol restored the expression of PPARγ. As expected, the treatment of ovariectomised female *Seipin*$^{+/-}$ mice with rosiglitazone restored their insulin synthesis and secretion, and these responses were accompanied by an improvement in glucose intolerance. When compared with intact female *Seipin*$^{+/-}$ mice, the levels of *Pdx-1*, *Nkx6.1*, *Glut2* and proinsulin mRNA in islet beta cells of ovariectomised *Seipin*$^{+/-}$ mice were markedly reduced; the reduced levels were recovered either by the activation of PPARγ or by oestrogen replacement. In addition, the action of oestradiol in beta cells can enhance insulin secretion by reducing ATP-sensitive potassium channel activity [44]. Therefore, oestrogen, acting through recovered expression of PPARγ, exerts

an important protective effect on insulin synthesis and secretion in female *Seipin*$^{+/-}$ mice.

The elevation of phosphatidic acid has been reported in seipin-knockdown yeast [7] and hippocampal neuronal cells of *Seipin*-knockout mice [45]. Thus, it has been proposed that absence of seipin may lead to the accumulation of phosphatidic acid [7], which may serve as a strong PPARγ antagonist [32] or cause the decline in PPARγ expression to downregulate the MAPK/ERK-CREB and Wnt3 signalling pathways [45, 46]. In addition, seipin physically interacts with sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) to regulate intracellular calcium homeostasis [47]. Moreover, seipin is required for the maintenance of lipid homeostasis [6]. Therefore, further studies are needed to determine the molecular mechanisms underlying the impaired insulin synthesis and secretion in *Seipin*-knockout islet beta cells.

In conclusion, heterozygous deletion of *Seipin* in islet beta cells reduces PPARγ expression, thereby causing a decline in insulin synthesis and secretion leading to glucose intolerance. These effects can be relieved by oestrogen treatment, which restores PPARγ expression. These findings provide new perspectives for the therapy of glucose intolerance in individuals with BSCL2.

Data availability All data generated or analysed during this study are included in this published article (and its ESM).

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement JX, PS, YaW, XH, WS and YanW contributed to acquisition, analysis and interpretation of data. PS and LC wrote the draft of the manuscript. JW, GL, WY and LC contributed to the conception and design of this work. All authors revised the manuscript critically for important intellectual content and approved the final version to be published. LC is the guarantor of this work.

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